Volume 160, number 1,2 FEBS 704 August 1983

Peptidyltransferase center of ribosomes

On the mechanism of action of alkaloid lycorine

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Received 7 June 1983

The molecular mechanism of action of the alkaloid lycorine has been revised. According to our results, lycorine inhibits the binding of CACCA-Leu⟵Ac to the donor site of the peptidyltransferase center of wheat-germ ribosomes, whereas the transpeptidation reaction in the system with the minimal model donor is not inhibited. The equilibrium constant of CACCA-Leu⟵Ac to the donor site of 80 S ribosomes is measured.

Alkaloid lycorine *80 S ribosome* *Mechanism of action* *Association constant* *Peptidyltransferase center*

1. INTRODUCTION

Some alkaloids of the *Amaryllidaceae* family (species *Narcissus, Lycoris, Clivia, Amaryllis, Ungernia* etc.) are known as inhibitors of protein synthesis catalyzed by eukaryotic ribosomes (rabbit reticulocytes, HeLa cells, *Saccharomyces cerevisiae* etc.) [1,2]. Narciclasine and lycorine, the most active inhibitors of this group of alkaloids, prevent the transfer of the *N*-acetylleucyl residue from CACCA-Leu⟵Ac onto puromycin [3,4]. It is postulated therefore that these alkaloids are inhibitors of the transpeptidation reaction [3,4].

It is shown here, that lycorine has no influence on the reaction of pA-Met⟵f with CACCA-Phe in the presence of pC catalyzed by wheat-germ ribosomes. At the same time lycorine inhibits selectively the binding of the pentanucleotide donor substrate CACCA-Leu⟵Ac to the donor site of the PTC of ribosomes.

Abbreviations: PTC, the peptidyltransferase center; CACCA-Phe and CACCA-Leu⟵Ac, 3′-terminal fragments of Phe-tRNA and AcLeu-tRNA, respectively; pA-Met⟵f, 3′(2′)-*O*-(*N*-formylmethionyl)-adenosine 5′-phosphate, the minimal model donor; pC, cytidine 5′-phosphate

2. MATERIALS AND METHODS

Ribosomes from wheat germ were isolated as in [5]. The 60 S subunits were a kind gift of Professor A. Legocki. CACCA-[^{14}C]Phe and CACCA-[^{14}C]Leu were prepared from *E. coli* B total tRNA aminoacylated by [^{14}C]phenylalanine, specific radioactivity 315 mCi/mmol and [^{14}C]leucine, 240 mCi/mmol (UVVVR, Czechoslovakia) or 354 mCi/mmol (Amersham), correspondingly. Both preparations were hydrolyzed by ribonuclease T_1 (Worthington, USA) and isolated on Whatman 3MM paper by electrophoresis, 3500 V, 100 mA, 2 h. Then CACCA-[^{14}C]Leu was acetylated with Ac_2O into CACCA-[^{14}C]Leu⟵Ac. pA-Met⟵f was synthesized according to [6].

The reaction of CACCA-[^{14}C]Leu⟵Ac with puromycin was done as in [7]; that of pA-Met⟵f with CACCA-[^{14}C]Phe as in [8]; adsorption of CACCA-[^{14}C]Phe as in [9]; CACCA-[^{14}C]Leu⟵Ac as in [10]. The experimental condi-

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tions are shown in the legends to figures. The yield of the reaction of pA-Met←f with CACCA-[^{14}C]Phe was measured after alkaline hydrolysis of the incubation mixture and extraction of fMet-[^{14}C]Phe into ethyl acetate as in [8].

3. RESULTS

3.1. *Catalysis of the transpeptidation reaction with wheat germ 80 S ribosomes and 60 S subunits with involvement of the minimal donor*

Fig.1 shows the time-dependence of the reaction of pA-Met←f with CACCA-[^{14}C]Phe in the presence of either wheat germ 80 S ribosomes or 60 S subunits. For comparison the same data are given for *E. coli* 70 S ribosomes. As follows from fig.1 both 80 S ribosomes and 60 S subunits catalyzed the reaction with pA-Met←f, the subunits being more active than ribosomes.

Fig.2 shows the dependence of reaction product yield against minimal donor concentration for 60 S subunits and 70 S ribosomes without pC and in its presence. As one can see, pC stimulates 3–4-fold the transpeptidation in the case of 60 S subunits and 4–5-fold in the case of 70 S ribosomes.

The data in fig.1 and 2 demonstrate that both wheat germ 80 S ribosomes and 60 S subunits catalyzed the transpeptidation reaction with pA-Met←f to the same extent as ribosomes from rat liver [11] and *E. coli* [8].

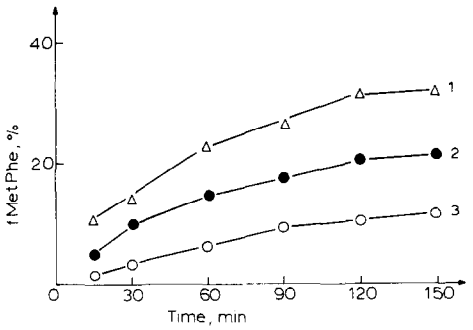

Fig.1. Time dependence of the reaction of pA-Met←f with CACCA-[^{14}C]Phe catalyzed by: *E. coli* 70 S ribosomes (1); wheat germ 60 S subunits (2); and 80 S ribosomes (3). The incubation mixture contained: CACCA-[^{1C}]Phe 71.4 pmol (= 100%); 70 S ribosomes (62.5 pmol); 60 S subunits (50.6 pmol); or 80 S ribosomes (82.3 pmol).

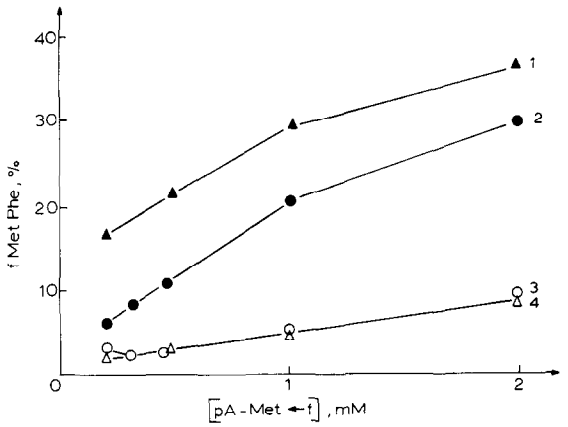

Fig.2. The yield dependence of the reaction of pA-Met←f with CACCA-[^{14}C]Phe *vs* pA-Met←f concentration in the presence of 1 mM pC (1,2) or without pC (3,4), catalyzed by 70 S ribosomes (1,3) or 60 S subunits (2,4). The incubation mixture contained: 70 S ribosomes (62.5 pmol); 60 S subunits (60 pmol); CACCA-[^{14}C]Phe 48.2 pmol (= 100%).

3.2. *The inhibition of reaction of CACCA-[^{14}C]Leu←Ac with puromycin by alkaloids*

Fig.3 shows the effect of lycorine, tazettine and ungerine on the reaction of CACCA-

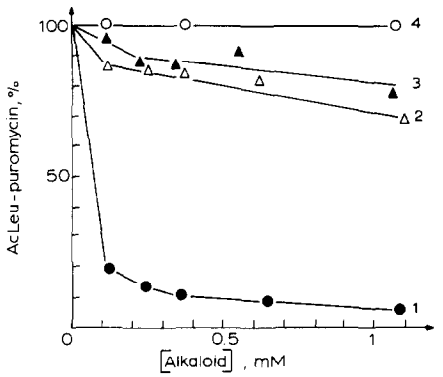

Fig.3. The action of the alkaloids lycorine (1), tazettine (2) and ungerine (3) on the CACCA-[^{14}C]Leu←Ac reaction with puromycin, catalyzed with wheat-germ 80 S ribosomes and the effect of lycorine on the reaction catalyzed with *E. coli* 70 S ribosomes (4). Incubation mixture contained: CACCA-[^{14}C]Leu←Ac 54.5 pmol; 70 S ribosomes 2.5 A_{260} units; 80 S ribosomes 5.2 A_{260} units. The yield of Ac-[^{14}C]Leu-puromycin without alkaloid corresponded to 1×10^4 cpm for 80 S ribosomes and 2×10^4 cpm for 70 S ribosomes. These data were taken to be 100%.

[^{14}C]Leu←Ac with puromycin catalyzed by ribosomes from wheat germ and *E. coli* MRE-600. One can see that lycorine effectively inhibited this reaction catalyzed by wheat germ ribosomes and had no effect on *E. coli* ribosomes. Two other alkaloids inhibited this reaction with wheat germ ribosomes to a much smaller degree. The data obtained in this experiment are similar to those shown for the ribosomes from rabbit reticulocyte and yeast [3,4].

3.3. *The effect of alkaloids on the reaction of pA-Met←f with CACCA-[^{14}C]Phe catalyzed by wheat germ ribosomes*

The influence of lycorine, tazettine and ungerine on the reaction of pA-Met←f with CACCA-[^{14}C]Phe in the presence of pC are shown in fig.4. One can see that tazettine and ungerine inhibited slightly, and lycorine did not inhibit the reaction. Fig.5 demonstrates the influence of lycorine on the reaction at different concentrations of pC. It was noticed that lycorine stimulated two times the transpeptidation without pC and by 10–15% in the presence of 2–2.5 mM pC.

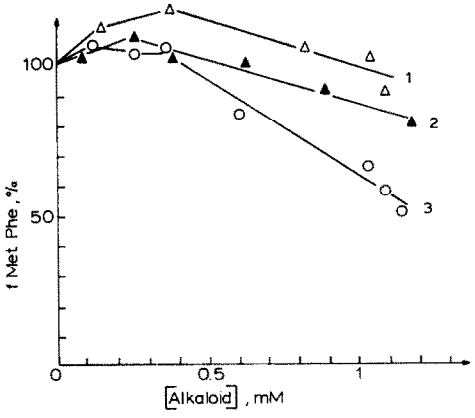

Fig.4. The effect of lycorine (1), ungerine (2) and tazettine (3) on the pA-Met←f reaction with CACCA-[^{14}C]Phe in the presence of 1.8 mM pC catalyzed with wheat-germ ribosomes. The incubation mixture contained CACCA-[^{14}C]Phe 37.5 pmol, 80 S ribosomes 3.9 A_{260} units. The yield of reaction products in the absence of alkaloid taken as 100% was 14.3 in the presence and 4.3 pmol in the absence of pC.

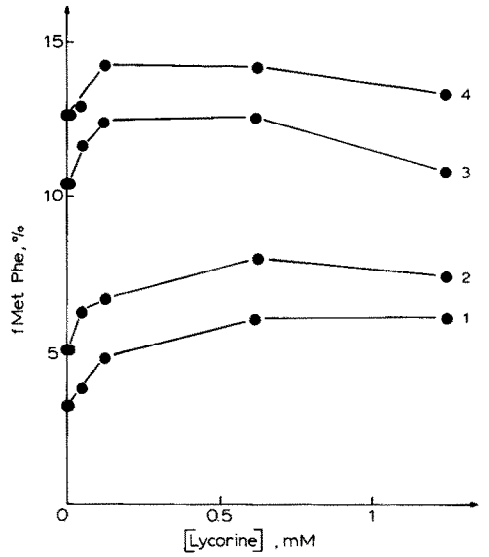

Fig.5. The effect of lycorine on the reaction of pA-Met←f with CACCA-[^{14}C]Phe catalyzed by 80 S ribosomes without pC (1) and in the presence of pC, 0.25 mM (2), 1.25 mM (3) and 2.5 mM (4). The assay mixture contained 80 S ribosomes 2.6 A_{260} units and CACCA-[^{14}C]Phe 30.7 pmol. The total radioactivity added was taken to be 100%.

3.4. *Effect of lycorine on the CACCA-[^{14}C]Phe and CACCA-[^{14}C]Leu←Ac binding to wheat germ ribosomes*

Fig.6 illustrates the absence of influence of lycorine on the binding of acceptor substrate CACCA-[^{14}C]Phe in the system without ethanol and a very slight inhibition of its binding in 40% ethanol.

Fig.7 shows the adsorption of CACCA-[^{14}C]Leu←Ac on 80 S ribosomes (curve 1) and the same adsorption in the presence of lycorine (curve 2) and gougerotin (curve 3). As one can see, there were two types of interaction of CACCA-Leu←Ac with 80 S ribosomes in the absence of both inhibitors. These interactions were characteristic of K_a 3.2 × 10^6 M^{-1} ($n_1 \approx 0.9$) and K_a 1.2 × 10^6 M^{-1} ($n \approx 1.1$). These findings demonstrate that the binding of CACCA-Leu←Ac occurs on two sites, the affinity being 2.5–3-times stronger for one of the sites.

The binding of CACCA-Leu←Ac in the presence of lycorine was observed for only one site, K_a 1.2 × 10^6 M^{-1}. As lycorine did not inhibit

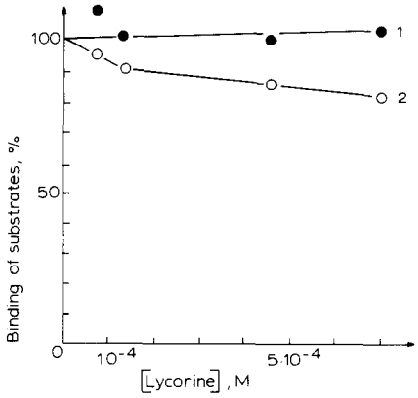

Fig.6. The binding of CACCA-[^{14}C]Phe to 80 S ribosomes in the presence of lycorine without ethanol (1) and in 40% ethanol (2). The assay mixture contained 80 S ribosomes 3.9 A_{260} units, CACCA-[^{14}C]Phe 44.6 pmol. The binding of CACCA-[^{14}C]Phe without ethanol and lycorine was achieved at 8.1 pmol and in ethanol at 16.0 pmol and taken to be 100%.

the binding of model substrate to the PTC acceptor site (fig.6), we believe that the above-mentioned binding reflects the interaction with the PTC acceptor site. An additional argument to be mentioned is that the binding of CACCA-Leu⟵Ac to ribosomes occurred in the presence of gougerotin. Gougerotin is a well-known inhibitor of the PTC acceptor site [12]. The adsorption of CACCA-Leu⟵Ac in this case is characterized by a strong affinity, K_a 1.02 × 10^7 M^{-1}, and therefore this adsorption takes place at the PTC donor site. As one can see from fig.7, lycorine inhibits effectively the adsorption of CACCA-Leu⟵Ac at the PTC donor site. The K_a of lycorine to ribosome was equal to 8.65 × 10^2 M^{-1}.

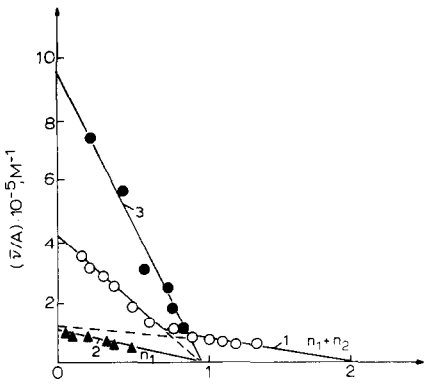

Fig.7. The binding of CACCA-[^{14}C]Leu⟵Ac to 80 S ribosomes (1), the same in the presence of lycorine, 1.5 mM (2) and gougerotin, 0.5 mM (3); ($\bar{\nu}$) the total portion of CACCA-[^{14}C]Leu⟵Ac capable of associating with ribosomes; (A) concentration of free CACCA-[^{14}C]Leu⟵Ac. The assay mixture contained 80 S ribosomes 16.6 pmol, ethanol 40% (v/v), the quantity of CACCA-[^{14}C]Leu⟵Ac varied from 6.65–224.16 pmol (1) or from 6.65–113.65 pmol (2,3).

The comparison of K_a of CACCA-Leu⟵Ac in the presence and absence of gougerotin (fig.7) shows that antibiotic increased the affinity of CACCA-Leu⟵Ac to the donor site nearly 2-fold.

4. DISCUSSION

According to the above-presented results lycorine does not inhibit peptide bond formation as unambiguously follows from the absence of inhibition of the pA-Met⟵f reaction with CACCA-[^{14}C]Phe (fig.4). Also lycorine has no influence on the CACCA-Phe, pA-Met⟵f and pC binding, which follows from the fact that the blocking of the above-mentioned reaction was not observed. The absence of inhibition of the CACCA-Phe binding was shown by an independent experiment (fig.6).

However, lycorine blocked the reaction of CACCA-[^{14}C]Leu⟵Ac with puromycin (fig.3). The mechanism of inhibition included the binding of lycorine to ribosome with K_a 8.65 × 10^2 M^{-1} and a subsequent blocking of the CACCA-Leu⟵Ac adsorption at the PTC donor site (fig.7). K_a of lycorine was determined from the equation [13]:

$$K_a = (K'_a - K''_a)/K''_a \times C$$

where:

K'_a = the association constant of CACCA-Leu⟵Ac without lycorine;
K''_a = that in the presence of lycorine;
C = lycorine concentration.

In fig.7, 90% of the population of 80 S ribosomes were active and homogeneous in adsorption tests with model substrates at the PTC site, K_a of CACCA-Leu⟵Ac being rather close to K_a of this compound at the PTC donor site of *E. coli* ribosomes [8].

The molecular mechanism of lycorine action is unknown so far. It can be suggested that after binding to 60 S ribosomal subunits [14] lycorine changes their conformation in the PTC region and these events can result in blocking the oligonucleotide model donor adsorption.

Attention should be drawn to the property of lycorine to block the binding of pentanucleotide donor substrate to the PTC donor site. Until now, a selective inhibitor of the PTC donor site for both prokaryotic and eukaryotic ribosomes has not been known. Lycorine appears to be the first reported agent of this type.

ACKNOWLEDGEMENTS

The authors are thankful to Dr Nagima Ajtkhozhina for her assistance in preparation of wheat germ ribosomes and to Dr Andrey Belikov for alkaloids. We are much obliged to Mrs Galina Moussinova for translation of the manuscript.

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